IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Andrea Piva

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DECLARATION OF ANDREA PIVA

Andrea Piva declares as follows:

1. I presently reside at Via De'Carbonesi 7, 40123 Bologna, ITALY.

2. I graduated in 1990 *cum laude* at Universita' Cattolica del S.Cuore, Piacenza, Italy in Agricultural Science, for which I received the Georgofili Award by the Italian President of the Minister Council in 1991. I defended my PhD in 1993 in Molecular Biotechnology with a joint program at the Biotechnology Research Center, Cremona, Italy, and at the Cell and Molecular Biology Group, Dept. of Biochemistry, University College, Galway, Ireland. I was assigned the position of Assistant Professor at Dept. of Morpho-Physiology and Animal Production (DIMORFIPA), School of Veterinary Medicine, University of Bologna, Italy, in 1992, Assistant Professor with tenure in 1995, Associate Professor at DIMORFIPA in 2000 and since 2004 Full Professor in animal nutrition at the School of Veterinary Medicine, University of Bologna, Italy.

3. I was a visiting scientist at the Food Research Institute, Dept. of Food Microbiology & Toxicology, University of Wisconsin, Madison, Wisconsin, from 1995 until 1999 as Italian Consiglio Nazionale delle Ricerche scholar, as a participant of the "Fulbright Program", sponsored by the United States Department of State, Bureau of Educational and Cultural Affairs, and of the Program "Short Term-Mobility 1998" of the Italian Consiglio Nazionale delle Ricerche. I was then a visiting scientist as participant of

the "Co-operative Research Program: Biological Resource Management for Sustainable Agricultural Systems", with the Organisation for Economic Co-operation and Development (OECD), at the Eastern Regional Research Center, Agricultural Research Service, USDA in 1999-2000.

4. My research activities are related to the antimicrobial activity of lactic acid bacteria, intestinal ecosystem and metabolism, and diet and intestinal mucosa interactions in food animals. I am the research leader of projects co-sponsored by the regional government and private companies in the field of alternative strategies to antibiotic growth promoters and in the field of food-born pathogens in the swine and poultry food chain as well as projects sponsored by the Italian MIUR (Ministry of Education, University and Research), relative to mycotoxins contamination and preventive strategies.

5. I have served as a reviewer for the following journals: *Livestock Production Science* (Official Journal of the European Association for Animal Production), *British Journal of Nutrition*, *Italian Journal of Animal Science*, *Agricultural and Food Science*, *Canadian Journal of Animal Science*, *Journal of Animal Physiology and Animal Nutrition*. I am an author or co-author of 163 publications, of which 46 were in peer-review journals. I am a co-inventor of three patents, being US Patent No. 6217915 (feed additive that increases availability of butyric acid and non-digestible oligosaccharides in the gastrointestinal tract); European Patent No. EP 1391155 B1 (composition for use in animal nutrition comprising a controlled release matrix, process for preparing and use thereof); and European Patent No. EP 2185005 A2 (synergetic composition comprising flavouring substances and organic acids and use thereof).

6. I am a co-inventor of the above-referenced US Patent Application No. 10/551,536. I am a majority owner of AVIP S.r.L., the assignee of the above-referenced US Application No. 10/551,536.

7. A key feature of the above-referenced Application No. 10/551,536 is that previously isolated Pediocin A is added to animal feed, which is then fed to an animal such as a swine or poultry. Pediocin A is a protein which is produced by the bacteria *Pediococcus pentosaceus* FBB61. Normally, it is expected that proteins such as Pediocin A would be degraded in the stomach of an animal, due to the low PH of the stomach and protease activity in the stomach. Because Pediocin A would be expected to be degraded in the stomach, it would not be expected to have any beneficial effects on the animal, since it would be degraded before it could reach the small and/or large intestines, where it could be active. However, it has been found that Pediocin A, when added to animal feed which is fed to an animal, surprisingly and unexpectedly increases the growth rate and provides significant beneficial effects on the growth of the animal. This has been shown in several studies, described as follows.

8. Attached hereto as Attachment A is an article entitled "Pediocin A Improves Growth Performance in Broilers Challenged with *Clostridium perfringens*", published in Poultry Science, Volume 88, pages 2152-2158 (2009). I am a co-author of this article and the studies therein were carried out under my supervision. The article describes two studies, a Pilot Study and a Floor Pen Study. In the Pilot Study, as described at page 2153, in the right hand column, halfway down, 36 female Ross 508 broilers (poultry) were divided into three groups and fed for 21 days as follows: a negative control (CTR) fed basal diet (Table 1); a positive control (the same diet supplemented with supernatant filtrate of a cultural broth of the isogenic mutant *P. pentosaceus* FBB61-2 devoid of pediocin A expression (Bac-); and a treated group, fed the control diet supplemented with supernatant filtrate of a cultural broth of *P. pentosaceus* FBB61 (Bac+). In the Bac+ group, pediocin A was provided at 80 AU/g of feed. The birds were challenged with 10^6 cells of *Clostridium perfringens* and fed ad libitum for 21 days.

9. In the Pilot Study, the presence of pediocin A in the animal feed surprisingly and unexpectedly increased the growth rate and improved the growth performance of the broilers. Data are shown in Table 2. During the first period (0 to 9 days), Average Daily Gain (ADG) was significantly higher for Bac+ versus CTR and Bac- (+31 and +21% vs.

CTR and Bac- , respectively; $P < 0.01$), and consequently, Body Weight (BW) was higher for Bac+ versus CTR and Bac- (+24 and +17% vs. CTR and Bac-, respectively; $P < 0.01$). On day 14, ADG was still higher for Bac+ compared with CTR (+23%, $P = 0.02$), and BW was higher for Bac+ and Bac- compared with CTR (+23 and +14%, respectively; $P = 0.02$). Feed Conversion Ratio (FCR) was better for Bac+ than for Bac- at 0-9 days (1.08 vs. 1.10) and at 0-14 days (1.29 vs. 1.38). FCR is the grams of feed needed to increase body weight by 1 gram; therefore, a lower FCR is better.

10. In summary, surprisingly and unexpectedly, the addition of pediocin A to the feed resulted in increased growth rate and improved growth performance for the broilers.

11. The Poultry Science article also discussed a second study, called the Floor Pen Study. In this study, described on page 2154, 216 Ross 508 broilers were divided into three experimental groups: a negative control group (CTR) fed the basal diet (Table 1); a group inoculated with *C. perfringens* (CP); and a group inoculated with *C. perfringens* and fed a diet supplemented with *P. pentosaceus* FBB61 (10^7 cfu/g) and pediocin A (PA); the pediocin A was provided at 60 and 40 AU/g feed in the first and second phase diets (0 to 14 days; 15 to 42 days), respectively. The growth performance of the Floor Pen Study is discussed on page 2155, right hand column, which states that the data is shown in Table 3. During the period 0 to 14 days, Average Daily Gain was significantly higher for PA versus CTR and CP (+14 and +15%, respectively; P = 0.01); and Feed Conversion Ratio tended to be numerically lower in PA versus CTR and CP (-23 and -20%, respectively; P= 0.08). At 14 days, the Body Weight was significantly higher for PA versus CTR and CP (+17 and +18%, respectively; P < 0.01). In the second phase, Average Daily Gain of PA tended to be higher compared with the CP group (+4%, P = 0.08) and equal to the unchallenged birds. As can be seen, surprisingly and unexpectedly, the poultry fed feed supplemented with a combination of pediocin A and its producer strain *P. pentosaceus* FBB61 had increased growth rate and improved growth performance.

12. Attached as Attachment B are two screens summarizing a third study carried out under my supervision where a first group of 24 piglets was fed a diet including *P. pentosaceus* FBB61 cultured broth containing pediocin A and *P. pentosaceus* FBB61 bacteria (Bac+). A second group of 24 piglets was fed the same diet supplemented with *P. pentosaceus* FBB61-2 (Bac-) cultured broth, containing FBB61-2 bacteria but no pediocin A, since FBB61-2 is non-producing for pediocin A. In other words, the diets were the same except that the diet of the Bac+ piglets contained pediocin A (at a concentration of 40 AU/g of feed) while the diet of the Bac- piglets did not contain pediocin A. The second page of Attachment B shows the results of the study. As can be seen, after 14 days, the ADG for the Bac+ piglets is 155, while for the Bac- piglets it is 138, and the feed conversion ratio (FCR) for the Bac+ piglets is 1.98, whereas for the Bac- piglets, it is 2.18. For days 0 to 35, the ADG for the Bac+ piglets is 409, while for the Bac- piglets it is 387, and the feed conversion ratio (FCR) is 1.58 for the Bac+ piglets and 1.68 for the Bac- piglets. For days 14 to 35, the ADG for the Bac+ piglets is 578, while for the Bac- piglets it is 554, and the feed conversion ratio (FCR) is 1.52 for the Bac+ piglets and 1.61 for the Bac- piglets. In summary, it can be seen that the results of the piglet study are similar to the results of the poultry study reported in Poultry Science. Surprisingly and unexpectedly, this piglet study showed that the addition of pediocin A to animal feed resulted in increased growth rate of the piglets. The results of the piglet study are surprising and unexpected, because it would be expected that the pediocin A protein would be degraded in the stomach and not be available to produce any growth enhancing benefits to the piglet.

13. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C.

1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: __July 15, 2010____ Signature: _____/andreapiva/_____
ANDREA PIVA